Claims

1. A method of detecting and analyzing protein interactions in a cell, which comprises the steps:
   a) provision of the activity of at least one enzyme from the group consisting of recombinases and proteases in the cell as a result of a protein interaction,
   b) continual generation of an active reporter protein in the cell in question as a result of the enzymic activity of step a) for a period of time which exceeds the duration of the protein interaction of step a),
   c) generation of a detection signal by the reporter proteins generated in b).

2. The method as claimed in claim 1, characterized in that the active reporter protein in step b) is continually generated in the cell in question for such a period of time which comprises the entire lifetime of the cell in question.

3. The method as claimed in claim 2, characterized in that the reporter protein is additionally generated in the daughter cells of the cell in question in such a way that the entire lifetime of said daughter cells is encompassed.

4. The method as claimed in any of claims 1 to 3, characterized in that the continual generation of the active reporter protein according to step b) results in its accumulation in the cell in question or its daughter cells, said accumulation generating a detection signal which is increased compared to a detection signal generated by a reporter protein generated only for the duration of the protein interaction.

5. The method as claimed in any of claims 1 to 4, characterized in that the protein interaction to be detected or to be analyzed is a stimulus-induced protein interaction of a transient nature.

6. The method as claimed in any of claims 1 to 5, characterized in that the enzyme of step a) is a recombinase whose activity is provided by transfecting or infecting the cells with the expression vector i) which comprises a recombinase gene under the control of a transcription factor,
   and in that the continual generation of the active reporter protein in the cell in question according to step b) is carried out by the individual steps:

b1) transfection or infection of the cell with a construct ii) which comprises a stop cassette flanked by recombination sites with the downstream nucleotide sequence of a reporter gene under the control of a constitutive promoter,

b2) removal of said stop cassette of construct ii), flanked by recombination sites, by means of the recombinase of a),

b3) constitutive expression of the reporter gene.

7. The method as claimed in claim 6, characterized in that the transfections or infections of the cell are stable.

8. The method as claimed in claim 6, characterized in that the transfections or infections of the cell are transient.

9. The method as claimed in any of claims 6 to 8, characterized in that the reporter gene used is a gene from the group consisting of fluorescent reporter genes, reporter genes with enzymic function, resistance-imparting genes and reporter genes for growth selection.

10. The method as claimed in claim 9, characterized in that the reporter gene used is the green fluorescent protein (GFP), any of its variants, luciferase or β-galactosidase.

11. The method as claimed in any of claims 1 to 5, characterized in that the enzyme of step a) is a recombinase whose activity is provided by the protein interaction-dependent transcomplementation of a functional recombinase in the nucleus, and in that the continual generation of the active reporter protein in the cell in question according to step b) is carried out by the individual steps:

b4) transfection or infection of the cell with a construct ii) which comprises a stop cassette flanked by recombination sites with the downstream nucleotide sequence of a reporter gene under the control of a constitutive promoter,

b5) removal of said stop cassette of construct ii), flanked by recombination sites, by means of the recombinase of a),

b6) constitutive expression of the reporter gene.

12. The method as claimed in claim 11, characterized in that transcomplementation of the functional recombinase is carried out by the steps:

d1) expression of a first fusion protein comprising the first interaction partner and part of the recombinase and of a second fusion protein comprising the second interaction partner and another part of the recombinase,

d2) reconstitution of a functional recombinase due to said first and second interaction partners interacting with one another in the nucleus.

13. The method as claimed in any of claims 1 to 5, characterized in that the activities of a protease and of a recombinase are provided and in that the activity of said protease arises from protein interaction-dependent transcomplementation of a functional protease.

14. The method as claimed in claim 13, characterized in that transcomplementation of the functional protease is carried out by the steps:

e) expression of

e1) a first fusion protein comprising the first interaction partner and part of a protease, and

e2) a second fusion protein comprising the second interaction partner and another part of said protease,

with, where appropriate, at least one of the two fusion proteins possessing a further domain which causes said fusion protein to be anchored outside the nucleus, and

e3) expression of a functional recombinase,
which, where appropriate, is a further domain of the first or second fusion protein and can be proteolytically removed from the other domains via a recognition and cleavage site for the protease, or expression of a functional recombinase
on a third fusion protein which, in addition to the functional recombinase itself which is proteolytically removable via a recognition and cleavage site for the protease, comprises a further domain causing the third fusion protein to be anchored outside the nucleus,

in a cell,

f) reconstitution of a functional protease due to the first and second interaction partners interacting with one another,

g) proteolytic removal of the functional recombinase from its anchoring position outside the nucleus by the reconstituted protease of f),

h) transport of the functional recombinase into the nucleus and activation of a recombinase-dependent reporter gene.

15. The method as claimed in claim 14, characterized in that the component e3) expressed in step e) is a functional recombinase which possesses at least one further domain which causes said recombinase to be anchored on the cell membrane and which is proteolytically removed in step g) from its anchoring position on the cell membrane.

16. The method as claimed in any of claims 1 to 5, characterized in that the activities of a protease and of a recombinase are provided and in that a protein interaction-imparted spatial proximity between said protease and its substrate is generated.

17. The method as claimed in claim 16, characterized in that the activity of the protease in the cell due to spatial proximity between said protease and its substrate is generated by the steps:

j) expression of

j1) a first fusion protein comprising the first interaction partner and a functional protease, and

j2) a second fusion protein comprising the second interaction partner, a functional recombinase domain and a further domain causing anchoring outside the nucleus, with at least said functional recombinase domain being proteolytically removable from the domain which causes the second fusion protein to be anchored outside the nucleus, via a recognition and cleavage site of the protease used,

in the cell,

k) effecting a spatial proximity, resulting from the interaction of the first and the second interaction partner, between the functional protease of the first fusion protein and the protease recognition and cleavage site on the second fusion protein,

l) proteolytically removing the functional recombinase anchored outside the nucleus by cleaving the protease cleavage site with the proximal protease,

transporting the free functional recombinase into the nucleus and activating a reporter system.

18. The method as claimed in any of claims 1 to 5, characterized in that the reporter protein of step b) is provided in the cell in question by, as a direct or indirect result of protein interaction, a specific functional transcription factor which induces or increases expression of said reporter protein being provided in the nucleus of said cell.

19. The method as claimed in claim 18, characterized in that the specific functional transcription factor in the nucleus of the cell is provided by protein interaction-dependent transcomplementation of a functional transcription factor in said nucleus.

20. The method as claimed in claim 19, characterized in that transcomplementation of the functional transcription factor comprises the steps:

m) expression of a first fusion protein comprising the first interaction partner and part of the transcription factor and of a second fusion protein comprising the second interaction partner and another part of the transcription factor,

n) reconstitution of a functional transcription factor due to said first and second interaction partners interacting with one another,

o) induction of expression of a functional recombinase for activation of a recombinase-dependent reporter system in the nucleus.

21. The method as claimed in claim 18, characterized in that the functional transcription factor is provided by protein interaction-imparted proximity between a protease and its substrate.

22. The method as claimed in claim 21, characterized in that the spatial proximity between the protease and its substrate is generated by the steps:

j) expression of

j1) a first fusion protein comprising the first interaction partner and a functional protease, and

j2) a second fusion protein comprising the second interaction partner, a functional transcription factor domain and a further domain causing anchoring outside the nucleus, with at least said functional transcription factor domain being proteolytically removable from the domain which causes the second fusion protein to be anchored outside the nucleus, via a recognition and cleavage site of the protease used,

in the cell,

k) effecting a spatial proximity, resulting from the interaction of the first and the second interaction partner, between the functional protease of the first fusion protein and the protease recognition and cleavage site on the second fusion protein,

l) proteolytically removing the functional transcription factor anchored outside the nucleus by cleaving the protease cleavage site with the proximal protease, transporting the free functional transcription factor into the nucleus and activating a reporter system.

23. The method as claimed in claim 18, characterized in that the transcription factor is provided by protein interaction-dependent transcomplementation of a protease.

24. The method as claimed in claim 23, characterized in that transcomplementation of the protease is achieved by the steps:

p) expression of

p1) a first fusion protein comprising the first interaction partner and part of a protease, and

p2) a second fusion protein comprising the second interaction partner and another part of said protease,

with, where appropriate, at least one of the two fusion proteins possessing a further domain which causes the fusion protein to be anchored outside the nucleus, and

p3) expression of a functional transcription factor,

which, where appropriate, is a further domain of the first or second fusion protein and which is proteolytically removable from the other

domains of said fusion protein via a recognition and cleavage site for a protease, or

expression of a functional transcription factor on a third fusion protein which, in addition to the functional transcription factor itself which is proteolytically removable via a recognition and cleavage site for said protease, comprises a further domain which causes said third fusion protein to be anchored outside the nucleus;

in a cell;

q) reconstitution of a functional protease due to the first and second interaction partners interacting with one another;

r) proteolytically removing the functional transcription factor from its anchoring position outside the nucleus by the reconstituted protease of q)

s) providing a functional transcription factor in the nucleus and subsequently inducing expression of a recombinase-dependent or a recombinase-independent classical reporter system.

25. The method as claimed in claim 24, characterized in that the functional transcription factor proteolytically removed in step r) causes in step s) in the nucleus induction of expression of a recombinase-independent classical reporter system and additionally causes induction of the expression of a functional protease for further continual activation of the reporter system employed.

26. A method of detecting and analyzing protein interactions in a cell, which comprises the steps:

u) expression of

u1) a first fusion protein comprising the first interaction partner and part of a protease, and

u2) a second fusion protein comprising the second interaction partner and another part of said protease, and

u3) a reporter which can be activated or inactivated by proteolysis,

in the cell,

v) reconstitution of a functional protease due to said first and second interaction partners interacting with one another,

w) activation of the proteolysis-activatable or inactivation of the proteolysis-inactivatable reporter by the reconstituted functional protease of step v).

27. The method as claimed in claim 26, characterized in that the component u3) expressed in step u) is a proteolysis-activatable reporter protein whose reporter activity has been inactivated by insertion of an additional amino acid sequence which is flanked on one side or on both sides by at least one recognition site and/or cleavage site for a protease.

28. The method as claimed in claim 26, characterized in that the component u3) expressed in step u) is a proteolysis-inactivatable reporter protein which contains at least one recognition and cleavage site for a protease and whose reporter activity can be proteolytically inactivated.

29. A method of detecting and analyzing protein interactions in a cell, which comprises the steps:

x) expression of

x1) a first fusion protein comprising the first interaction partner and a functional protease, and

x2) a second fusion protein comprising the second interaction partner and a reporter which can be activated or inactivated by proteolysis,

in the cell;

y) interaction of said first and second interaction partners with one another;

z) activation of the proteolysis-activatable reporter or inactivation of the proteolysis-inactivatable reporter by protein interaction-dependent spatial proximity of the functional protease of the first fusion protein x1) and the proteolysis-activatable or -inactivatable reporter of the second fusion protein x2).

30. The method as claimed in any of claims 1 to 5, characterized in that the enzyme of step a) is a protease and in that continual generation of the active reporter protein in the cell in question according to step b) is carried out by the individual steps:

A) expression of

A1) a first fusion protein comprising the first interaction partner and part of a protease, and

A2) a second fusion protein comprising the second interaction partner and another part of said protease, and

A3) a protease which can be activated by proteolysis,

in the cell,

B) transcomplementation of a functional protease due to said first and second interaction partners interacting with one another,

C) activation of the proteolytically activatable proteases by the transcomplemented functional protease of step B),

D) activation or inactivation of a proteolytically activatable or a proteolytically inactivatable reporter system by the functional proteases of steps B) and C).

31. A method of detecting and analyzing protein interactions in a cell, which comprises the steps

J) expression of

J1) a first fusion protein comprising the first interaction partner and part of a protease, and

J2) a second fusion protein comprising the second interaction partner and another part of said protease, and

J3) constitutive expression of a reporter protein which is anchored via a suitable domain outside the nucleus and which can be proteolytically removed from said anchoring position, and additionally comprises a further domain which, after proteolytic removal, effects localization of said reporter protein into a particular compartment of the cell,

in the cell,

K) reconstitution of a functional protease due to said first and second interaction partners interacting with one another,

L) proteolytically removing the reporter protein together with its domain which causes localization of the free reporter protein into a particular compartment of the cell, by the functional protease of step K),

M) detecting the altered location of the reporter protein.

32. A screening method of identifying a specific interaction partner of a defined protein by carrying out the method as claimed in any of claims 1 to 31.

33. The screening method as claimed in claim 32, characterized in that a specific interaction partner of the defined protein is identified by expressing a cDNA library or an ORF library.

34. The screening mehod as claimed in either of claims 32 and 33, characterized in that it is used for identifying substances which influence, in particular inhibit or activate, a specific protein interaction.

35. A method of detecting and analyzing the disassociation of a defined protein interaction in a cell, which comprises the steps:

P) provision of the activity of at least one enzyme from the group consisting of recombinases and proteases in the cell as a result of the dissociation of a protein interaction,

Q) continual generation of an active reporter protein in the cell in question as a result of the enzyme activity of step P) for a period of time which exceeds the duration of the dissociated state of the protein interaction,

R) generation of a detection signal by the reporter proteins generated in Q).

36. The method as claimed in claim 35, characterized in that the active reporter protein in step Q) is generated for such a period of time which comprises the entire lifetime of the cell in question.

37. The method as claimed in claim 36, characterized in that the active reporter protein in step Q) is additionally generated in the daughter cells of the cell in question in such a way that the entire lifetime of said daughter cells is encompassed.

38. The method as claimed in any of claims 35 to 37, characterized in that continual generation of the active reporter protein according to step Q) results in the accumulation of said active reporter protein in the cell in question or its daughter cells, said accumulation generating a detection signal which is increased compared to such a detection signal which is increased compared to a detection signal generated by a only for the duration of the dissociation of the protein interaction.

39. The method as claimed in any of claims 35 to 38, characterized in that the dissociation to be detected or analyzed of a protein interaction is a stimulus-induced dissociation of a transient nature.

40. The method as claimed in any of claims 35 to 39, characterized in that dissociation of the protein interaction is caused by at least one of the events selected from:
the presence of a specific inhibitor of said protein interaction, and
the presence of a stimulus influencing said protein interaction.

41. The method as claimed in any of claims 35 to 40, characterized in that the enzyme of step P) is a recombinase and in that continual generation of the active reporter protein in the cell in question according to step Q) is carried out by the individual steps:

S) expression and specific interaction of
   S1) a first fusion protein comprising the first interaction partner and a functional recombinase, and
   S2) a second fusion protein comprising the second interaction partner and an inhibitor of said recombinase,
   in the cell,

T) induced dissociation of the interacting fusion proteins, thereby removing the proximity between the recombinase and its inhibitor and providing a functional recombinase,

U) activation of a recombinase-dependent reporter system by the functional recombinase of step T).

42. The method as claimed in any of claims 35 to 40, characterized in that the enzyme of step P) is a recombinase and in that continual generation of the active reporter protein in the cell in question according to step Q) is carried out by providing, as a result of dissociation of the defined protein interaction, in the nucleus of said cell a specific functional transcription factor which induces or increases expression of a recombinase, said recombinase subsequently activating a recombinase-dependent reporter gene.

43. The method as claimed in any of claims 35 to 40, characterized in that continual generation of the active reporter protein in the cell in question according to step Q) is carried out by the steps:

V) expression and specific interaction of
   V1) a first fusion protein comprising the first interaction partner and a functional protease, and
   V2) a second fusion protein comprising the second interaction partner and an inhibitor for said functional protease, and
   with, where appropriate, at least one of the two fusion proteins possessing a further domain resulting in the anchoring of the fusion protein outside the nucleus, and

V3) expression of a functional protein selected from the group consisting of transcription factors, recombinases and proteolytically activatable or inactivatable reporter proteins,
which protein, where appropriate, is a further domain of the first or the second fusion protein and which is proteolytically removable from the remaining domains by a recognition and cleavage site for the protease,
or which, where appropriate, is a further constitutively expressed fusion protein and a domain for anchoring outside the nucleus and which is proteolytically removable from its anchoring position via a recognition and cleavage site for said protease,

V4) where appropriate, expression of a proteolytically activatable protease,
in the cell,

W) induced dissociation of the interacting fusion proteins, thereby removing the proximity between the protease and its inhibitor and providing a functional protease,

X) proteolytically removing the functional recombinase or the functional transcription factor of V3) from its anchoring position outside the nucleus by the functional protease of step W) and transport into the nucleus,

Y) activation of a recombinase-dependent reporter system or proteolytic activation of the proteolytically activatable protease of V4) by the functional protease of step W),

Z) activation or inactivation of the proteolytically activatable or inactivatable reporter proteins of V3) by the functional proteases of step W) and of step Y).

44. The method as claimed in claim 43, characterized in that the functional protein V3) expressed in step V) is a functional transcription factor proteolytically removable from its anchoring position outside the nucleus, which transcription factor is proteolytically removed in step X) by the functional protease of step W) and which activates in step Y) a recombinase-dependent reporter system.

45. The method as claimed in claim 43, characterized in that a further functional protein V3) expressed in step V) is a functional recombinase proteolytically removable from its anchoring position outside the nucleus, which recombinase is

removed in step X) from its anchoring position by the functional protease of step W) and which activates in step Y) a recombinase-dependent reporter gene.

46. The method as claimed in claim 43, characterized in that the functional protein V3) expressed in step V) is a proteolytically activatable or inactivatable reporter protein and in that the proteolytically activatable protease V4) is expressed which is proteolytically activated in step Y) by the functional protease of step W) and which activates or inactivates in step Z) a proteolytically activatable or inactivatable reporter protein.

47. A screening method of identifying or characterizing specific inhibitors or activators of defined protein interaction or of identifying or characterizing a defined stimulus influencing a defined protein interaction, carrying out the method as claimed in any of claims 35 to 46.

48. A cell which has been transfected or infected with at least one expression vector, the latter comprising at least one, preferably at least two, in particular at least three, of the constructs i) to vii), and with

i) a construct comprising a recombinase gene being under the control of a transcription factor

ii) a construct comprising a stop cassette flanked by recombination sites with the downstream nucleotide sequence of a reporter gene under control of a constitutive promoter,

iii) a construct comprising a recombinase which is anchored outside the nucleus and which can be proteolytically removed,

iv) a construct comprising a transcription factor which is anchored outside the nucleus and which can be proteolytically removed,

v) a construct comprising a proteolytically activatable or inactivatable reporter protein,

vi) a construct comprising a proteolytically activatable protease,

vii) a protease gene under the control of a transcription factor.

49. The cell as claimed in claim 48, characterized in that said cell has been stably transfected or infected.

50. The cell as claimed in claim 48, characterized in that said cell has been transiently transfected or infected.

51. The cell as claimed in any of claims 48 to 50, characterized in that said cell is selected from the group consisting of bacterial cells, yeast cells or cells of higher eukaryotes, in particular neuronal cells or mammalian cell lines.

52. A kit for detecting and analyzing protein interactions in a cell, which comprises the expression vectors comprising the nucleic acid constructs 1a) and 2a), in each case under the transcriptional control of a heterologous promoter:

1a) a first nucleic acid construct coding for a first fusion protein comprising the nucleic acid sequence coding for a first protease fragment and a cloning site suitable for cloning the bait protein in the reading frame of the first protease fragment, and

2a) a second nucleic acid construct coding for a second fusion protein comprising the nucleic acid sequence coding for a second protease fragment and a cloning site suitable for cloning the prey protein in the reading frame of the second protease fragment,

and which, where appropriate, comprises expression vectors comprising at least one of the following constructs:

3a) a nucleic acid construct coding for a functional recombinase or for a functional transcription factor which can be proteolytically removed via a recognition and cleavage site for a protease from a domain for anchoring to a cytoplasmic structure, it being possible for said components to be used either as further moieties of the first or second fusion protein or as separate third fusion protein;

4a) a nucleic acid construct coding for a recombinase under the control of the functional transcription factor of No. 3;

5a) a nucleic acid construct comprising a stop cassette flanked by recognition sites for recombinases with the downstream nucleotide sequence of a reporter gene under the control of a constitutive promoter;

6a) a nucleic acid construct coding for a proteolytically activatable or proteolytically inactivatable reporter protein under the control of a promoter;

7a) a nucleic acid construct coding for a proteolytically activatable protease under the control of a promoter.

53. A kit for detecting and analyzing protein interactions in a cell, which comprises expression vectors comprising the nucleic acid constructs 1b) and 2b), in each case under the transcriptional control of a heterologous promoter:

1b) a first nucleic acid construct coding for a first fusion protein comprising the nucleic acid sequence coding for a functional protease and a cloning site suitable for cloning the first interaction partner into the reading frame of said functional protease, and

2b) a second nucleic acid construct coding for a second fusion protein comprising

the nucleic acid sequence coding for a protein selected from the group consisting of recombinases, transcription factors and reporter proteins,

a cloning site suitable for cloning the second interaction partner into the reading frame of said protein,

and, where appropriate, a nucleic acid sequence coding in the reading frame of said protein for a protein domain resulting in the second fusion protein being anchored outside the nucleus,

and, where appropriate, which comprises expression vectors which comprise at least one of the constructs:

3b) a construct for expressing a recombinase gene under the control of the functional transcription factor of No. 2;

4b) a construct which comprises a stop cassette flanked by recognition sites for recombinases with the downstream nucleotide sequence of a reporter gene under the control of a constitutive promoter.

54. A kit for detecting and analyzing protein interactions in a cell, which comprises expression vectors comprising at least one of the nucleic acid constructs 1c) and 2c), in each case under transcriptional control of a heterologous promoter:

1c) a first nucleic acid construct coding for a first fusion protein, comprising the nucleic acid sequence coding for a first part of a protein selected from the group consisting of transcription factors or recombinases and a cloning site suitable for cloning the first interaction partner into the reading frame of said protein,

2c) a second nucleic acid construct coding for a second fusion protein, comprising the nucleic acid sequence coding for a second part of a protein selected from the group of transcription factors or recombinases and a cloning site suitable for cloning the second interaction partner into the reading frame of said protein,

and which comprises, where appropriate, expression vectors comprising at least one of the following constructs:

3c) a construct for expressing a recombinase gene under the control of the functional, transcomplemented protein of No. 1 and 2, said protein being a transcription factor;

4c) a construct comprising a stop cassette flanked by recognition sites for recombinases with the downstream nucleotide sequence of a reporter gene under the control of a constitutive promoter.

55. A kit for detecting and analyzing protein interactions in the cell, which comprises at least one expression vector comprising at least one of the nucleic acid constructs 1d) and 2d), in each case under the transcriptional control of a heterologous promoter:

1d) a first nucleic acid construct coding for a first fusion protein comprising a functional enzyme from the group consisting of proteases or recombinases and a bait protein, and

2d) a second nucleic acid construct coding for a second fusion protein comprising a functional inhibitor for an enzyme of the group consisting of proteases and recombinases and a prey protein.

56. The kit as claimed in any of claims 52 to 54, characterized in that a cDNA library for screening for interaction partners of the bait protein has been cloned into the cloning site of the second fusion protein according to 2a), 2b) and 2c).

57. The kit as claimed in any of claims 52 to 56, characterized in that additionally cells selected from the group consisting of bacterial cell, yeast cell or cells of higher eukaryotes, in particular neuronal cells or mammalian cell lines, are provided, which cells can be transfected or infected with the expression vectors defined in claims 52 to 55.

58. The kit as claimed in claim 57, characterized in that the additionally provided cells are transfected or infected with at least one of the expression vectors defined in claims 52 to 54 and in that only the expression vectors, defined in claims 52 to 55, with which said cells have not been transfected or infected are additionally provided in the form of expression plasmids.

59. The use of at least one enzyme from the group consisting of recombinases and proteases or of at least one expression vector encoding such an enzyme for continual generation of an active reporter protein in a cell for a period of time exceeding the duration of protein interaction..